



Characterization of *Mucor racemosus* lipase with potential application for the treatment of cellulite

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ABSTRACT

An extracellular lipase LII from *Mucor racemosus* was purified by anion exchange and gel filtration chromatography using DEAE-Sepharose and Sephacryl S-200 columns, respectively. A molecular weight of 20 kDa was estimated for *M. racemosus* LII by Sephacryl S-200 column and SDS-PAGE. LII had an acidic pI at 4.8. V_{max} and K_m for LII were estimated to be 55.5 μmol oleic acid/min/ml and 2% olive oil, respectively. The highest lipolytic activity was detected with linseed oil as the substrate. The optimal pH and temperature for LII was 5.0 and 40 °C, respectively. The enzyme was stable up to 70 °C. The lipase activity was strongly enhanced by Cu^{2+} , Al^{3+} and Fe^{3+} , and slightly enhanced by Mg^{2+} , Ni^{2+} and Mn^{2+} , whereas Ca^{2+} , Co^{2+} , K^+ and Hg^{2+} showed no effect. Pb^{2+} only caused a partial inhibition of the enzyme. The residual lipase activity in carbopol 934 base gel was 385% after storage at 4 °C for 420 days. The characteristic properties of *M. racemosus* lipase formulated in the carbopol 934 gel was utilized for topical treatment of cellulite, and the results were positive for reducing thigh circumference.

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1. Introduction

Lipases (triacylglycerol acylhydrolase, E.C. 3.1.1.3.) are ubiquitous enzymes of considerable physiological significance and industrial potential. Lipases catalyze a wide range of reactions, including hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification and aminolysis. Therefore, lipases, especially microbial lipases have many industrial applications [1]. Following proteases and carbohydrases, lipases are considered to be the third largest group of enzyme based on total sales volume [2].

In the pharmaceutical industry, lipases are used as digestive enzymes, and also in diagnostic kits for blood triacylglycerol, pancreatic carcinoma and acute as well as chronic renal diseases, which are characterized by a rise in serum lipase [3]. Stereoselectivity of lipases has been used to resolve various racemic organic acid mixtures in immiscible biphasic systems [4]. The overwhelming interest for screening lipases for use in the cosmetic and perfume industry has mainly been due to its activity in surfactant and aroma production, which are the main ingredients in cosmetics and perfumes [5]. Monoacylglycerols and diacylglycerols, prepared by the lipase-catalyzed esterification of glycerol, are useful surfactants in

cosmetics. Mixed-acid-type polyester and acylglycerol ester fatty acids are the main components in lipase-mediated cosmetics [6].

Cellulite is the unsightly dimpling and nodularity frequently found on the thighs and buttocks of post-adolescent women. It is estimated that 85% of postpubertal women have some degree of cellulite [7,8]. The appearance of cellulite is probably caused by conformational changes in fibrous septae within the hypodermis that leads to herniation of subcutaneous fat into the dermis [9]. Excess subcutaneous fat should not be confused with obesity where only adipocyte hyperplasia and hypertrophy occur. Thus, cellulite is more complex and involves the presence of excess subcutaneous fat, the microcirculatory system, lymphatics and the extracellular matrix. Fibroblasts activated by estrogen are thought to play a special role in the pathogenesis of cellulite by increasing glycosaminoglycan synthesis and leading to increased interstitial osmotic pressure as well as tissue fluid retention. Therefore, small blood vessels become compressed, provoking tissue hypoxia, inflammation, elevated inflammatory cytokine levels and increased collagen synthesis. Increased capillary pressure and increased differences between interstitial and capillary osmotic pressure lead to intercellular edema [10,11]. Cellulite is not specific to overweight females although increased body mass will exacerbate the condition. Weight loss itself may ameliorate cellulite severity, but the skin surface does not dramatically change, histological analyses suggest that fat globules protrude from the dermis with weight loss [12].

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